

What is claimed is:

1. A chimeric protein which comprises a mutated DNA methyltransferase portion and a DNA binding protein portion that binds sufficiently close to a promoter sequence of a target gene, which promoter sequence contains a methylation site, to specifically methylate the site and inhibit activity of the promoter and thus inhibit expression of the target gene.
2. The protein of claim 1, wherein the promoter sequence of the target gene is a 5' long terminal repeat sequence of a human immunodeficiency virus-1 proviral DNA.
3. The protein of claim 1, wherein the target gene comprises a retroviral gene, an adenoviral gene, a foamy viral gene, a parvoviral gene, a foreign gene expressed in a cell, an overexpressed gene, or a misexpressed gene.
4. The protein of claim 1, wherein the chimeric protein comprises a zinc three-finger DNA binding polypeptide linked to a CpG-specific DNA methyltransferase polypeptide.
5. The protein of claim 1, wherein the chimeric protein comprises a mutated Lex A binding polypeptide linked to a cytosine methyltransferase polypeptide.
6. The method of claim 1, wherein the mutated DNA methyltransferase portion comprises at least a portion of a mutated *M.SssI* DNA methyltransferase protein or at least a portion of a mutated mammalian DNA methyltransferase protein.

-70-

7. An expression vector which encodes the chimeric protein of claim 1.
8. The vector of claim 7, wherein the expression vector is replicable.
9. The vector of claim 7, wherein the vector is a pLS vector.
10. The vector of claim 7, wherein the vector is a prokaryotic expression vector, a yeast expression vector, a baculovirus expression vector, a mammalian expression vector, or an episomal mammalian expression vector.
- 
11. A method for inhibiting expression of a target gene which comprises contacting a promoter of the target gene with the chimeric protein of claim 1 so as to specifically methylate the promoter thus inhibiting expression of the target gene.
12. The method of claim 11, wherein the target gene is an endogenous target gene.
- 
13. The method of claim 11, wherein the target gene is a foreign target gene.
14. The method of claim 13, wherein the foreign target gene is a retroviral gene or a viral gene.
- 
15. The method of claim 11, wherein the target gene is associated with a cancer, a central nervous system disorder, a blood disorder, a metabolic disorder,

866007 "ETOF5060

Sum  
C3

MD5

Sum  
C4

-71-

*Sub  
C4  
cont*

a cardiovascular disorder, an autoimmune disorder, or an inflammatory disorder.

---

16. The method of claim 15, wherein the cancer is acute lymphocytic leukemia, acute myelogenous leukemia, B-cell lymphoma, lung cancer, breast cancer, ovarian cancer, prostate cancer, lymphoma, Hodgkin's disease, malignant melanoma, neuroblastoma, renal cell carcinoma or squamous cell carcinoma.
17. The method of claim 15, wherein the central nervous system disorder is Alzheimer's disease, Down's syndrome, Parkinson's disease, Huntington's disease, schizophrenia, or multiple sclerosis.
18. The method of claim 15, wherein the infectious disease is cytomegalovirus, herpes simplex virus, human immunodeficiency virus, AIDS, papillomavirus, influenza, candida albicans, mycobacteria, septic shock, or associated with a gram negative bacteria.
19. The method of claim 15, wherein the blood disorder is anemia, hemoglobinopathies, sickle cell anemia, or hemophilia.
20. The method of claim 15, wherein the cardiovascular disorder is familial hypercholesterolemia, atherosclerosis, or renin/angiotensin control disorder.
21. The method of claim 15, wherein the metabolic disorder is ADA, deficient SCID, diabetes, cystic fibrosis, Gaucher's disease, galactosemia, growth hormone deficiency, inherited emphysema, Lesch-

000510130000

-72-

Nyhan disease, liver failure, muscular dystrophy, phenylketonuria, or Tay-Sachs disease.

22. The method of claim 15, wherein the autoimmune disorder is arthritis, psoriasis, HIV, or atopic dermatitis.

23. The method of claim 15, wherein the inflammatory disorder is acute pancreatitis, irritable bowel syndrome, Chron's disease or an allergic disorder.

24. The method of claim 11, wherein the target gene is in a cell.

25. The method of claim 24, wherein the cell is a eukaryotic cell, a bacterial cell, an animal cell, a plant cell, a prokaryotic cell, a virus packaging cell, a somatic cell, a germ cell, a neuronal cell, a myocyte, a T lymphocyte, a CD4<sup>+</sup> cell, a tumor cell, a CD4<sup>+</sup> cell, or a stem cell.

26. The method of claim 11, wherein the contacting is by means of liposome mediated delivery, retroviral delivery, gene bombardment, electroporation or cationic precipitation.

27. A method for inhibiting expression of a target gene in a multicellular organism which comprises contacting a promoter sequence of the target gene with the chimeric protein of claim 1, so as to specifically methylate the promoter sequence and thus inhibit expression of the target gene in the multicellular organism.

B6600T ETOT5060

-73-

28. The method of claim 27, wherein the multicellular organism is a plant, an animal or a human.
29. The method of claim 28, wherein the plant is an alfalfa plant, a broccoli plant, a rapeseed plant, a carrot plant, a chicory plant, a coffee plant, a cucurbita plant, a euromelon plant, a potato plant, a raspberry plant, a sunflower plant, a tomato plant, or a wheat plant.
30. The method of claim 28, wherein the animal is a horse, a primate, a porcine animal, a bovine animal, a swine, a fowl, or a fish.
31. The method of claim 27, wherein the chimeric protein or a nucleic acid encoding the chimeric protein is delivered to the multicellular organism via intralesional, intraperitoneal, intramuscular or intravenous injection; liposome-mediated delivery; viral infection; gene bombardment; topical, nasal, oral, anal, ocular or otic delivery.
32. The method of claim 31, wherein the viral infection is via a non-integrating, replication-defective virus.
33. The method of claim 32, wherein the virus comprises a replication-defective Human Immunodeficiency Type 1 provirus, a retroviral vector, an adeno-associated virus, a LNL6 vector, a LXSXN vector or a MMuLV retroviral vector.
34. A method of treating a subject infected with a virus which comprises administering to the subject

866001 E F015060

-74-

a therapeutic composition comprising the chimeric protein of claim 1, or a nucleic acid molecule encoding the chimeric protein of claim 1, under suitable conditions so as to specifically methylate the viral promoter sequence and inhibit expression of the viral gene thus treating the subject infected with the virus.

35. The method of claim 34, wherein the virus is chosen from the group consisting of a DNA virus, a retrovirus, a herpes virus, an immunodeficiency virus, an adeno-associated virus and an adenovirus.
36. The method of claim 34, wherein the therapeutic composition comprises a nucleic acid molecule encoding a mutated Lex A DNA binding protein portion linked to a mutated DNA methyltransferase protein portion.
37. The method of claim 34, wherein the therapeutic composition comprises a nucleic acid molecule encoding a tridactyl zinc finger DNA binding protein portion capable of specifically binding the Human Immunodeficiency Virus Type 1 5' long terminal repeat nucleic acid sequence linked to a mutated DNA methyltransferase protein portion.
38. The method of claim 34, wherein the subject is a human.
39. The method of claim 34, wherein the therapeutic composition comprises a replicable expression vector chosen from the group consisting of a pLS vector, a prokaryotic expression vector, a yeast expression vector, a baculovirus expression vector,

B6600T\* EFTF5060

-75-

a mammalian expression vector, and an episomal mammalian expression vector.

40. The method of claim 34, wherein the administration comprises intralesional, intraperitoneal, intramuscular or intravenous injection; liposome-mediated delivery; viral infection; gene bombardment; topical, nasal, oral, anal, ocular or otic delivery.
41. The method of claim 40, wherein the viral infection is via a non-integrating, replication-defective virus.
42. A host cell comprising the expression vector of claim 7.
43. The host cell of claim 42, wherein the host cell is chosen from the group consisting of a eukaryotic cell, a somatic cell, a germ cell, a neuronal cell, a myocyte, a T lymphocyte, a prokaryotic cell, a virus packaging cell, a plant cell, a prokaryotic cell, a tumor cell, a stem cell and a CD4+ cell.
44. A pharmaceutical composition comprising a therapeutically effective amount of the expression vector of claim 7 and a pharmaceutically acceptable carrier.
45. The pharmaceutical composition of claim 44, wherein the carrier comprises a diluent.
46. The pharmaceutical composition of claim 44, wherein the pharmaceutically acceptable carrier is an aerosol, intravenous, oral or topical carrier.

0905101.10093B

-76-

47. A transgenic non-human mammal whose somatic and germ cells contain and express a DNA coding for a chimeric protein of claim 1, the DNA having been stably introduced into the non-human mammal at the single cell stage or an embryonic stage, and wherein the DNA is linked to a promoter and integrated into the genome of the non-human mammal.

03051013-10093  
866007-ET05060